J. Anat. (1994), 185, pp. 135–142, with 3 figures Printed in Great Britain

Growth of secondary hair follicles of the Cashmere goat in vitro and their response to prolactin and melatonin

MAJID IBRAHEEM[1], HUGH GALBRAITH[1], JEREMY SCAIFE[1] AND STANLEY EWEN[2]

Departments of [1] Agriculture and [2] Pathology, University of Aberdeen, UK

(*Accepted 20 January 1994*)

ABSTRACT

The isolation and viability in vitro of anagen secondary hair follicles of the Cashmere goat were studied. Isolated hair follicles were used to determine the effects on hair shaft elongation, of prolactin and melatonin, hormones considered to influence hair follicle growth and activity in vivo. Intact hair follicles were isolated from the dermal layer of the skin singly or in groups using watchmakers' forceps under a dissecting microscope. The isolated follicles were maintained floating in Williams E medium. The medium was supplemented with 1 of 6 concentrations of ovine prolactin (0, 50, 200, 400, 800 and 4000 μg/l) for the culture of hair follicles isolated during July and August, and with 1 of 5 concentrations of melatonin (0, 50, 150, 300, 600 ng/l) for the culture of hair follicles isolated during September and October. There was clear evidence of DNA synthesis, observed by autoradiography, in matrix cells of freshly isolated follicles incubated for 6 h in the presence of [methyl-^{3}H]-thymidine. Similar measurements after 96 h of maintenance indicated a marked reduction in the incorporation of [methyl-^{3}H]-thymidine in matrix cells of the follicles studied. Prolactin and melatonin were shown to have a stimulating effect on hair shaft elongation of secondary follicles during 24 h periods of measurement and cumulatively over 120 h. Maximum hair follicle growth was observed in follicles exposed to 400 μg/l of prolactin and follicles exposed to 300 ng/l of melatonin. The number of follicles remaining viable during each 24 h measuring period was not affected by prolactin, but was significantly reduced by melatonin treatment after 96 h of maintenance. Hair follicle growth was significantly greater in July/August than September/October. The results for the in vitro method used suggest that prolactin and melatonin may act directly on the Cashmere secondary hair follicle to stimulate elongation of the hair shaft and that melatonin may reduce the viability of follicles after 96 h incubation. The results are discussed in the context of the possible involvement of prolactin and melatonin in the seasonal control of the hair growth cycle in vivo.

Key words: Tissue culture; autoradiography; photoperiodicity.

INTRODUCTION

The presence of a hair coat acts to protect mammals against environmental events including seasonal changes in climate. Many mammalian species of the temperate zone express photoperiodic-dictated alterations in coat growth which modify its insulating capability in order to prepare the animal for changes in ambient temperature. Hair growth in mammals is cyclical. At the end of the active (anagen) phase, the follicle passes through a phase of regression (catagen) into a resting (telogen) phase (Chase, 1954). Duration of anagen is constant while duration of telogen can be affected by photoperiod manipulation and hormones (Rougeot et al. 1984).

In common with other mammals such as sheep, and mink, many breeds of goat have a double coat consisting of the overhairs or guard hair produced by primary hair follicles and the underhairs or down (named cashmere commercially) produced by the secondary hair follicles (Burns et al. 1962). Cashmere fibre produced by such 'Cashmere' goats is usually no more than 6 cm in length, up to 0.018 mm in diameter, and is generally obscured by the outer coarse hair

Correspondence to Dr Hugh Galbraith, Department of Agriculture, University of Aberdeen, 581 King Street, Aberdeen, AB9 1UD, UK.

(Ryder, 1987). In contrast, the hair coat of the Angora goat consists essentially of a single fleece of mohair grown by secondary hair follicles with limited production of guard hairs from primary follicles (Dreyer & Marincowitz, 1967).

Photoperiodism is the main proximate factor in the control of seasonal coat change in Cashmere goats. Mitotic activity in the bulb of secondary hair follicles of the Cashmere goat is reported to be high from the summer solstice towards the winter solstice when it declines (Henderson & Sabine, 1990). In the spring, moulting occurs and both the primary and secondary follicles shed their fibres and a sparse coat of mainly guard hair is maintained until follicle activity starts again (Ryder, 1966; Nixon et al. 1991). Also, photoperiodism has been implicated in the control of seasonal coat changes in sheep (Lincoln et al. 1980), mink (Bissonnette & Wilson, 1939), red deer (Webster & Barrell, 1985), the horse (Burkhardt, 1947), and cattle (Yeates, 1955).

The pineal gland and pituitary glands have been shown to play an integral role in the mediation of the effects of photoperiodism on fibre growth and moulting periods in mammals. Allain et al. (1986) observed that the relationships between moulting periods and photoperiodic variation are suppressed by pinealectomy in sheep. A similar observation was made by Lincoln et al. (1980) following cervical sympathectomy in the Soay ram.

In the Cashmere goat, as in other mammals, the administration of melatonin (secreted by the pineal gland) has been shown to suppress the secretion of prolactin by the pituitary gland (Maeda et al. 1988). Exogenous melatonin administered by implantation in Cashmere goats has been shown to alter the normal pattern of fibre growth by inducing the growth of secondary hair follicles in the spring. As a consequence, the growth of the autumn coat was delayed as was the time when maximum length of fibre was achieved and when shedding occurred (Betteridge et al. 1987; Welch et al. 1990). Also, Gebbie et al. (1992) working in Cambridge, UK, have reported that oral administration of melatonin (3 mg per day for 8 wk from 8 March) suppressed the rise in prolactin and delayed the onset of the spring moult. However, when they administered the dopamine agonist, 2-bromo-α-ergocriptine (5 mg i.m. daily for 8 wk), prolactin secretion was suppressed without a corresponding delay in moulting. Other workers in the UK have reported a delaying effect of 2-bromo-α-ergocriptine on moulting (Lynch & Russel, 1992).

The mechanisms by which melatonin and prolactin affect hair growth cycle are largely unknown. The lack of knowledge is partly due to the absence of reliable in vitro models for the culture of hair follicles which would allow the direct investigation of hair follicle behaviour. Recently, we have adapted a tissue culture technique, developed originally for the culture of human hair follicles (Philpott et al. 1990), to the isolation and maintenance of secondary hair follicles of the Angora goat (Ibraheem et al. 1993). The objectives of the current study were to extend the above technique to the isolation and maintenance of intact anagen secondary hair follicles of the Cashmere goat and to investigate the effects of prolactin and melatonin on the pattern of growth of these follicles.

MATERIALS AND METHODS

Animals

Skin samples from 10 castrate male Cashmere (Siberian Don Altai) goats, aged 1 y, and kept locally (latitude 57° N) were used in this study.

Material

Williams E medium (without glutamine), phosphate buffered saline (PBS) and all tissue culture glassware were purchased from Gibco BRL Co. (Paisley, UK). Insulin, melatonin, hydrocortisone, L-glutamine, penicillin, streptomycin and thymidine were purchased from Sigma Chemical Co. (Dorset, UK). The [methyl-^{3}H] thymidine was obtained from Amersham Life Sciences (Buckinghamshire, UK) and K5 dipping emulsion from Ilford Co. (Mobberley, UK). Falcon 24-well multiwell plates were purchased from Becton Dickinson Co. (Oxford, UK).

Isolation and culture of hair follicles

Initial studies on the development of the method and response to prolactin were conducted in June to August 1992 when the anagen phase of follicle growth, occurring around and following the summer solstice, was expected to predominate. Subsequent studies (September and October 1992) investigated the effects of melatonin when anagen follicles were still present. One skin sample from each goat was taken immediately postmortem and the dermal layer was removed and examined with a dissecting microscope as described previously (Ibraheem et al. 1993). Using watchmakers' forceps to grip the external surface of the outer root sheath, intact secondary hair follicles were isolated by gently pulling them from the dermal

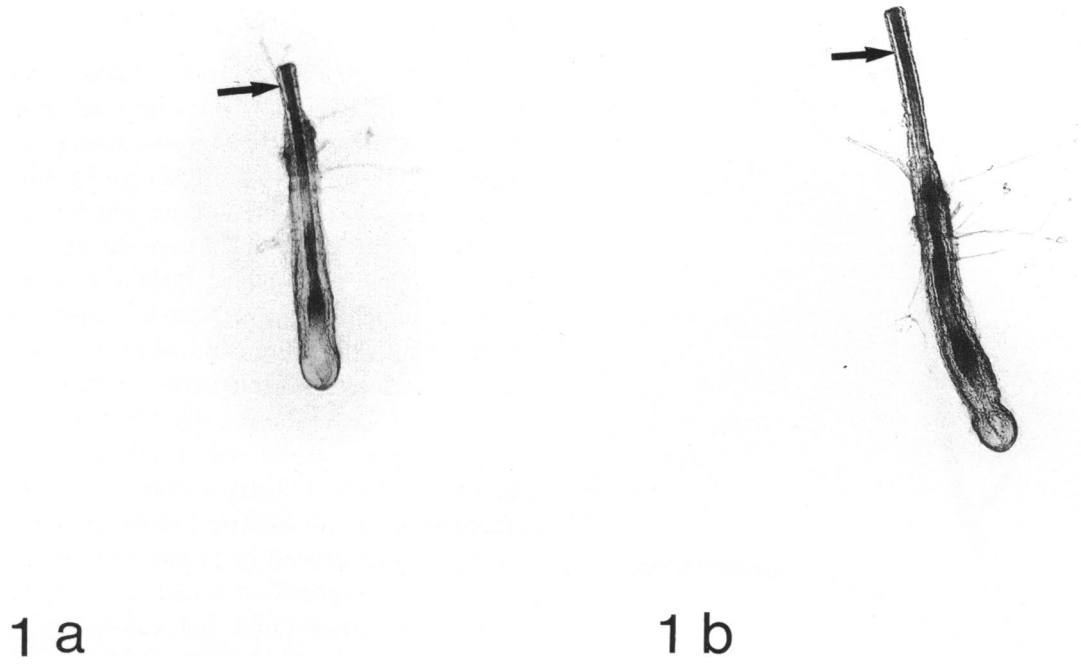

Fig. 1. Light micrographs showing the sequential elongation of the hair shaft (arrow) of a single hair follicle over 96 h in vitro. After 24 h of maintenance (*a*), after 96 h maintenance (*b*). ×40.

layer. Isolated hair follicles were maintained in 1.0 ml of Williams E medium supplemented with 1 mM L-glutamine, insulin (10 mg/l), hydrocortisone (10 μg/l), penicillin (64 mg/l) and streptomycin (100 mg/l). Isolated hair follicles were left free-floating in individual wells of multiwell plates at 37 °C in an unhumidified atmosphere of 5% CO_2/95% air. Follicles were maintained throughout the period of incubation in the medium in which they were placed at 0 h. Measurements of the changes in length of the follicles due to hair shaft elongation were made using a Microtech 200 inverted binocular microscope supplied with an eyepiece-measuring graticule. The minimum detectable change in length that could be measured was 0.02 mm. Follicles were considered to be nonviable if they did not elongate by at least 0.02 mm within each 24 h time period.

Autoradiography

The autoradiographic procedure was based on the method of Caro & van Tubergen (1962) and was previously described (Ibraheem et al. 1993).

Prolactin

The medium was supplemented at 0 h with one of 6 concentrations of ovine prolactin (NIDDK-oPRL-19, NIH, Baltimore, MD, USA) (0, 50, 200, 400, 800 and 4000 μg/l).

Melatonin

The medium was supplemented at 0 h with one of 5 concentrations of melatonin (0, 50, 150, 300 and 600 ng/l).

Statistical analysis

The statistical methods employed to analyse the results of all experiments were carried out using the BMDP statistical computer package (Dixon, 1983). Results are presented as treatment means with pooled standard error of the difference (S.E.D.). One-way analysis of variance was used followed by Student's t test to determine significant differences between group means.

RESULTS

Follicle preparation

With this dissecting technique it was possible to isolate 70–80 secondary follicles within 2 h from small 3 × 10 mm strips of the skin. A general view of a single follicle isolated from the dermis of the skin of a 1-y-old Cashmere goat, after 24 h in culture is shown in Figure 1 *a*. The bulb of the follicle appears to be intact and to have suffered no mechanical damage. Microscopic examination of this isolated follicle showed that it had increased in length due to hair shaft

Fig. 2. A light micrograph showing the elongation of the hair shaft (arrow) of 2 follicles after 120 h maintenance in Williams E medium supplemented with 200 μg/l prolactin ×40.

elongation. A representative photograph of the same growing follicle at 96 h is given (Fig. 1*b*) showing further increase in hair shaft elongation. Figure 2 shows the large elongation of the hair shaft of 2 follicles after 120 h of maintenance in Williams E medium supplemented with 200 μg/l prolactin.

Autoradiography

Synthesis of DNA was evident in freshly isolated follicles studied 6 h after incubation in the presence of [methyl-^{3}H] thymidine. There was an apparent marked reduction in DNA synthesis in the epidermal matrix cells of follicles exposed to [methyl-^{3}H] thymidine after 96 h of maintenance, with additional labelling evident in the adjacent outer root sheath (Fig. 3).

Prolactin and hair follicle growth

Increases in hair shaft length (means and pooled S.E.D.) of the follicles which expressed elongation of the hair shaft at each 24 h measuring period, and cumulatively over 120 h are shown in Table 1. The results indicate that the follicles which were exposed to the concentrations of 200 and 400 μg/l of prolactin showed significantly higher rates of hair shaft elongation cumulatively over 120 h maintenance than follicles not exposed or exposed to 50 or 4000 μg/l of prolactin. Over the same period, follicles exposed to 800 μg/l prolactin showed significant increases in hair shaft elongation compared to follicles not exposed to prolactin. Hair follicles cultured in the medium supplemented with 4000 μg/l prolactin showed significantly greater growth at 24 and 48 h of culture than follicles not exposed to prolactin. The exposure of follicles to 50 μg/l did not cause any significant increase in hair shaft elongation. There was a general trend for hair follicles exposed to concentrations of 200 μg/l of prolactin and above to show superior rates of growth throughout the first 72 h of culture compared to follicles not exposed to prolactin. There were no differences in viability (V, defined as the number of growing follicles at each measuring period) of the follicles across treatments.

Melatonin and hair follicle growth

Increases in hair shaft length (means and pooled S.E.D.) of the follicles which expressed growth at each 24 h measuring period, and cumulatively over 120 h are shown in Table 2. The results indicate that the growing follicles which were exposed to the concentrations of 150, 300, 600 ng/l of melatonin showed significantly higher rates of hair shaft elongation cumulatively over 120 h of maintenance than follicles not exposed to melatonin. No significant differences were observed between treatments after 24 h of culture. The pattern of growth of hair follicles exposed to 50 ng/l of melatonin did not differ significantly from the other treatments through culture. Follicles exposed to 300 ng/l of melatonin showed significantly higher growth rate at 48, 72 and 120 h after culture than follicles not exposed to melatonin. Follicles exposed to 150 and 600 ng/l of melatonin showed higher growth rates throughout culture than follicles not exposed to melatonin but these rates were significant only at 48 and 72 h respectively.

The number of growing follicles (V) at each measuring period declined more rapidly in the melatonin exposed follicles than the control. There

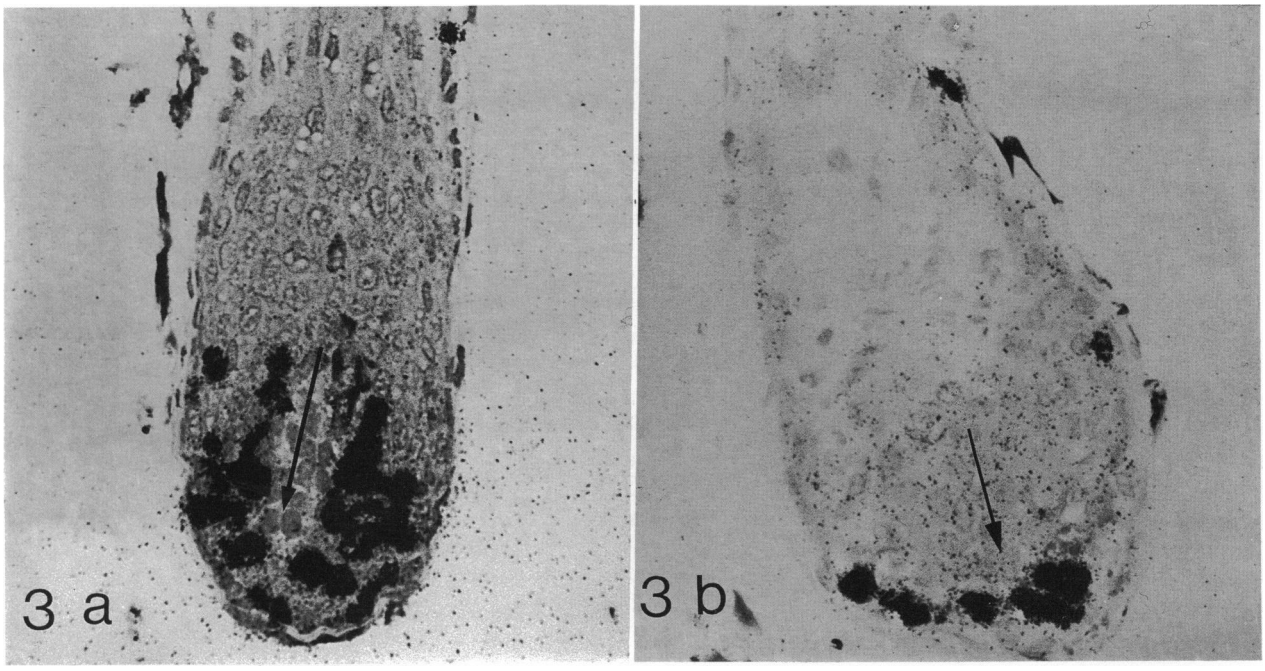

Fig. 3. Autoradiograph of [methyl-^{3}H] thymidine uptake by matrix cells adjacent to the dermal papilla (arrow) in a longitudinal section of a freshly isolated follicle (*a*), and the uptake by matrix cells in a similar section of a follicle maintained for 96 h in culture (*b*). Haematoxylin × 400.

Table 1. *Elongation (mm) and number of growing hair follicles (V) in 24 h growing periods and cumulatively over 120 h following exposure to prolactin in vitro (n = 5 Cashmere goats, 60 follicles examined/treatment)*

	Prolactin (μg/l)						
Hours	0	50	200	400	800	4000	S.E.D.
24	0.14^{a}	0.15^{c}	0.20^{b}	0.17^{abc}	0.18^{abc}	0.19^{bc}	0.013
V	55	57	53	52	55	52	1.76
48	0.14^{a}	0.16^{c}	0.20^{b}	0.18^{bc}	0.19^{bc}	0.19^{bc}	0.011
V	47	50	49	46	45	51	1.80
72	0.12^{a}	0.13^{ab}	0.16^{ab}	0.17^{b}	0.13^{ab}	0.12^{a}	0.013
V	43	40	45	41	44	40	1.98
96	0.13^{a}	0.14^{a}	0.14^{a}	0.16^{a}	0.13^{a}	0.12^{a}	0.013
V	36	37	32	34	35	33	2.08
120	0.14^{ab}	0.14^{ab}	0.14^{ab}	0.17^{b}	0.15^{b}	0.10^{a}	0.012
V	22	18	23	19	17	21	1.92
Cumulative totals	0.67^{a}	0.72^{ac}	0.84^{b}	0.86^{b}	0.78^{bc}	0.72^{ac}	0.024

Values are means with pooled S.E.D. Values in the same row with different superscripts are significantly different ($P < 0.05$).

were significant differences in viability between follicles exposed to 150 ng/l of melatonin at 96 and 120 h after culture, 600 ng/l of melatonin at 96 h after culture, 300 ng/l of melatonin at 120 h after culture and follicles not exposed to melatonin (see Table 2).

Effect of daylength

Mean hair shaft elongation (120 h cumulative total) in follicles of the control group isolated in July and August was significantly greater ($P < 0.001$) than in control follicles isolated in the months of September and October (0.67 vs 0.43 mm, S.E.D. = 0.0172—see Tables 1 and 2).

DISCUSSION

The work described here is the first illustration of the successful isolation, maintenance, and growth in vitro of secondary hair follicles of the Cashmere goat. Previously, we were able to isolate anagen hair follicles from the skin of the Angora goat and report on their

Table 2. *Elongation (mm) and number of growing hair follicles (V) in 24 h growing periods and cumulatively over 120 h following exposure to melatonin in vitro (n = 5 Cashmere goats, 60 follicles examined/treatment)*

	Melatonin (ng/l)					
Hours	0	50	150	300	600	S.E.D.
24	0.10^{a}	0.10^{a}	0.12^{a}	0.11^{a}	0.11^{a}	0.006
V	54	56	50	56	53	1.82
48	0.09^{a}	0.10^{ab}	0.11^{ab}	0.12^{b}	0.12^{b}	0.007
V	48	46	44	41	40	2.26
72	0.08^{a}	0.09^{ab}	0.11^{b}	0.11^{b}	0.10^{ab}	0.007
V	39	38	37	35	34	2.05
96	0.08^{a}	0.08^{a}	0.09^{a}	0.10^{a}	0.09^{a}	0.007
V	33^{a}	28^{ab}	26^{b}	29^{ab}	24^{b}	1.66
120	0.07^{a}	0.08^{ab}	0.09^{ab}	0.10^{b}	0.08^{ab}	0.007
V	21^{a}	16^{ab}	15^{b}	14^{b}	17^{ab}	1.46
Cumulative totals	0.43^{ab}	0.45^{bc}	0.51^{c}	0.53^{c}	0.51^{c}	0.017

Values are means with pooled S.E.D. Values in the same row with different superscripts are significantly different ($P < 0.05$).

significant growth in vitro (Ibraheem et al. 1993) using a microdissection model developed originally for the isolation and maintenance of human hair follicles (Philpott et al. 1990). In our studies on both Angora and Cashmere goats, we have found that hair follicles are not positioned in the fat as is the case with human hair follicle but in the dermal layer where they are arranged in groups, normally consisting of three primary follicles and a varying number of secondary follicles (Margolena, 1974; Henderson & Sabine, 1991). While the hair coat of the Angora goat consists essentially of a single fleece of mohair grown by secondary hair follicles with limited production of hairs from primary hair follicles, the Cashmere goat has a double coat consisting of overhairs and underhairs produced by primary and secondary hair follicles respectively (Burns et al. 1962; Dreyer & Marincowitz, 1967). The 2 types of follicles were easily distinguished prior to dissection on the basis of external morphology.

In the present study, we were able to isolate a larger number of follicles (70–80 compared with 24 in our study on Angora) within 2 h from 3 x 10 mm strips of the skin and this has been achieved through the refinement of the technique and accumulation of expertise. The follicles were isolated intact and undamaged visually from the dermis singly or in groups of 2, 3 and 4.

The average value (e.g. 0.14 (range 0.07–0.18) mm/day, at 24 h) for the rate of elongation of Cashmere follicles was less than that recorded for mohair (0.2; range 0.07–0.4) mm/d; Ibraheem et al. 1993). These differences reflect the differences expected in vivo for the rate of hair shaft elongation between the 2 breeds of goats (e.g. 0.450–0.684 mm/d for cashmere (Rhind & McMillen, 1993)) and up to 0.8 mm/d for mohair (Margolena, 1974). Data in vivo are not available for the goats used in the present study but, based on the above information, the cashmere growth rates in vitro are probably less than those occurring in vivo. The changes reported in follicle length are similar to those reported by Philpott et al. (1992) in vitro for pelage hair follicles in the rat. An analysis of the coefficients of variation for values for hair shaft elongation for each of the control and hormonal treatment groups indicated a maximum variation of 18% which confirms the representative nature of the mean values obtained for the follicles within each group.

It was apparent that the numbers of 'viable' follicles which continued to grow in each 24 h measuring period declined, particularly after 72 h incubation. A similar, but much greater reduction in viability which occurred after 48 h, was observed in the in vitro system for rat hair follicles of Philpott et al. (1992). These workers suggested that contributing factors may be the trauma of isolation, or the lack of an essential supplement which results in the failure of follicles to elongate.

There was clear evidence of DNA synthesis in the matrix cells of the bulb as shown by autoradiography of [methyl-^{3}H] thymidine uptake following 6 h incubation of freshly isolated follicles. This result for the pattern of DNA synthesis is similar to that reported under similar conditions for the Angora secondary hair follicle (Ibraheem et al. 1993) and the human hair follicle (Philpott et al. 1990). The results obtained for autoradiography of a selected follicle following 96 h of maintenance indicated a marked reduction in the

presence of [methyl-^{3}H]-thymidine incorporation into matrix cells. There was also evidence of incorporation of [methyl-^{3}H]-thymidine into the layer of cells comprising the outer root sheath. The result for matrix cells is consistent with a reduction of cellular activity in a large number of follicles at 96 h resulting in the observed decreases in viability recorded between 96 and 120 h incubation in vitro.

This study provides clear evidence of a stimulating effect of both prolactin and melatonin on the growth of secondary hair follicles of the Cashmere goat which follows a seasonal pattern controlled by changes in daylength (Ryder, 1966). These changes involve mediation through the actions of prolactin and melatonin (Gebbie et al. 1992). Previous studies have shown that keeping Cashmere goats in continuous light (which would allow elevated concentrations of blood prolactin) increased the mean fibre length from 14.6 to 23.2 mm (Ryder, 1987). Also, Deaville & Galbraith (1992) reported an association between increased dietary protein, elevated prolactin concentration in blood and greater fibre growth in the Angora goat.

The shedding process in the Cashmere goat occurs after the follicles enter the resting phase of the growth cycle (Ryder & Stephenson, 1968). The administration of melatonin (orally, implantation) has been shown to delay this process of shedding and to stimulate hair follicle growth (Litherland et al. 1990; Gebbie et al. 1992). The above results suggest that under certain conditions both prolactin and melatonin may stimulate hair follicle growth in vivo. The results from the present study suggest that hair follicle stimulation in vitro may be achieved by direct action of those hormones on the follicle. Previous workers have suggested that one of the effects of melatonin in vivo is to suppress prolactin plasma concentrations and perhaps to influence hair growth by an indirect rather than direct action (Prandi et al. 1987; Maeda et al. 1988).

In the current study, maximum hair follicle growth has been observed in follicles exposed to 400 μg/l of prolactin and follicles exposed to 300 ng/l of melatonin. These concentrations are at the upper end of the reported physiological range circulating in the plasma of sheep and goats (Waldhauser & Wurtman, 1983; Prandi et al. 1988). Differences between the values for elongation of control follicles and these exposed to prolactin or melatonin tended to be less at 120 h than earlier in the incubation. The reasons for this are not clear and will require further investigation. Possible explanations include changes in the sensitivity of the follicles to the hormonal agents or the possibility of reductions in the concentrations of melatonin and prolactin in the incubation medium.

The data from the present report also suggest that hair follicle growth was significantly greater ($P < 0.001$) in the months of July/August (0.67 mm per 120 h) than September/October (0.43 mm per 120 h). This result is in agreement with previous studies which have shown that mitotic activity in the hair follicle bulb of the Cashmere goat follows a seasonal trend with maximum activity occurring around the summer solstice, continuing throughout July and August, and declining thereafter (Henderson & Sabine, 1990). Also, in our study prolactin treatment did not have any effect on the number of growing follicles (V) at each measuring period throughout culture while melatonin supplementation of the medium caused a decline in the number of growing follicles which was evident after 96 h in culture. A similar pattern of reducing growth activity towards the winter solstice is associated with an increasing exposure to melatonin in vivo. It is therefore possible that such long term exposure to melatonin may contribute to the inactivation of hair follicles in vivo (Litherland et al. 1990; O'Neill et al. 1992) such as that which occurred during the shorter exposure in the present study.

In conclusion, we were able to extend the application of our microdissection technique from Angora to cashmere-bearing goats, and were successful in the isolation and maintenance of anagen secondary hair follicles. Also, we demonstrated an apparent direct effect of both prolactin and melatonin on hair follicle growth which may confirm an involvement of both hormones in hair follicle growth patterns in vivo. Additional work will be required to elucidate further the actions of these and other hormones on hair follicle activity during the hair growth cycle.

ACKNOWLEDGEMENTS

We thank the National Institute of Diabetes and Digestive and Kidney Diseases and the National Hormone and Pituitary Program (Baltimore, Maryland, USA) for the generous gift of ovine prolactin. We are also indebted to Mr J. Struthers and T. W. Begg for the care of the animals, Dr I. A. Forsyth and Dr J. S. M. Hutchinson for helpful advice, Mr G. King for expert technical support and Mrs E. Watt for secretarial assistance.

REFERENCES

ALLAIN D, RAVAULT JP, PANARETTO BA, ROUGEOT J (1986) Effects of pinealectomy on photoperiodic control of hair follicle activity

in the Limousine ram: possible relationship with plasma prolactin levels. *Journal of Pineal Research* **3**, 25–32.

BETTERIDGE K, DEVANTIER BP, WELCH RAS, POMROY WE, LAPWOOD KP (1987) Out of season cashmere growth in feral goats. In *Proceedings of the 2nd International Cashmere Congress*, pp. 137–144. New Zealand: Lincoln College Press.

BISSONNETTE TH, WILSON E (1939) Shortening daylight periods between May 15 and September 12 and the pelt cycle in mink. *Science* **89**, 418–419.

BURKHARDT J (1947) Transition from anoestrus in the mare and the effect of artificial lighting. *Journal of Agricultural Science (Cambridge)* **37**, 64–68.

BURNS RH, BERGEN W VON, YOUNG SS (1962) Cashmere and the undercoat of domestic and wild animals. *Journal of Textile Institute* **53**, T54–68.

CARO LG, VAN TUBERGEN RP (1962) High-resolution autoradiography. *Journal of Cell Biology* **15**, 173–188.

CHASE HB (1954) Growth of the hair. *Physiological Reviews* **34**, 113–126.

DEAVILLE ER, GALBRAITH H (1992) Effect of dietary protein level and yeast culture on growth, blood prolactin and mohair fibre characteristics of British Angora goats. *Animal Feed Science and Technology* **38**, 123–133.

DIXON WJ (1983) *BMDP Statistical Software*, pp. 105–114. Berkeley: University of California Press.

DREYER JH, MARINCOWITZ G (1967) Some observations on the skin histology and fibre characteristics of the Angora goat (*Capra hircus angoraensis*). *South African Journal of Agricultural Science* **10**, 477–500.

GEBBIE FE, FORSYTH IA, ARENDT J (1992) Effect of exogenous melatonin or bromocriptine on moulting and prolactin in goats. *Journal of Reproduction and Fertility Abstract Series* **9**, 4.

HENDERSON M, SABINE JR (1990) Seasonal secondary activity in Cashmere-type goats. *Proceedings of the Australian Society of Animal Production* **18**, 486.

HENDERSON M, SABINE JR (1991) Secondary follicle development in Australian cashmere goats. *Small Ruminants Research* **4**, 349–363.

IBRAHEEM MF, GALBRAITH H, SCAIFE J, EWEN S (1993) Growth and viability of secondary hair follicles of the Angora goat cultured in vitro. *Journal of Anatomy* **182**, 231–238.

LINCOLN GA, KLANDORF H, ANDERSON N (1980) Photoperiodic control of thyroid function and wool and horn growth in rams and the effect of cranial sympathectomy. *Endocrinology* **107**, 1543–1548.

LITHERLAND AJ, PATERSON DJ, PARRY AL, DICK HB, STAPLES LD (1990) Melatonin for cashmere production. *Proceedings of the New Zealand Society of Animal Production* **52**, 339–343.

LYNCH P, RUSSEL AFJ (1992) The endocrine control of fibre growth with special reference to the Cashmere goat. *Goat Veterinary Society Journal* **11**, 26–32.

MAEDA KI, MORI Y, KANO Y (1988) Involvement of melatonin in the seasonal changes of the gonadal function and prolactin secretion in female goats. *Reproduction Nutrition Development* **28**, 487–497.

MARGOLENA LA (1974) Mohair histogenesis, maturation and shedding in the Angora goat. *United States Department of Agriculture Technical Bulletin* **1495.**

NIXON AJ, GURNSEY MP, BETTERIDGE K, MITCHELL RJ, WELCH RAS (1991) Seasonal hair follicle activity and fibre growth in some New Zealand Cashmere-bearing goats (Caprus hircus). *Journal of Zoology (London)* **224**, 589–598.

O'NEILL KT, LITHERLAND AJ, HAMILTON G (1992) Melatonin for cashmere production in breeding does. *Proceedings of the New Zealand Society of Animal Production* **52**, 161–164.

PHILPOTT MP, GREEN MR, KEALEY T (1990) Human hair follicle growth in vitro. *Journal of Cell Science* **97**, 463–471.

PHILPOTT MP, GREEN MR, KEALEY T (1992) Rat hair follicle growth in vitro. *British Journal of Dermatology* **127**, 600–607.

PRANDI A, ROMAGNOLI G, CHIESA F, TAMANINI C (1987) Plasma prolactin variations and onset of ovarian activity in lactating anoestrous goats given melatonin. *Animal Reproduction Science* **13**, 291–297.

PRANDI A, MOTTA M, CHIESA F, TAMANINI C (1988) Circannual rhythm of plasma prolactin in the goat. *Animal Reproduction Science* **17**, 95–94.

RHIND SM, MCMILLEN SR (1993) Rate of growth and timing of growth and moulting of secondary fibre in Siberian and Iceland × feral goats fed high and low levels of dietary protein. *Animal Production* **56**, 447. (Abstract.)

ROUGEOT J, THEBAULT RG, ALLAIN D (1984) Role of the compound hair follicle in adaptive pelage changes. *Acta Zoologica Fennica* **171**, 19–21.

RYDER ML (1966) Coat structure and seasonal shedding in goats. *Animal Production* **8**, 289–302.

RYDER ML (1987) *Cashmere, Mohair and Other Luxury Animal Fibres for the Breeder and Spinner*, pp. 1–23. Southampton: Itchen.

RYDER ML, STEPHENSON SK (1968) *Wool Growth*. London: Academic Press.

WALDHAUSER F, WURTMAN RJ (1983) The secretions and actions of melatonin. In *Biochemical Actions of Hormones*, pp. 187–225. London: Academic Press.

WEBSTER JR, BARRELL GK (1985) Advancement of reproductive activity, seasonal reduction in prolactin secretion and seasonal pelage changes in pubertal red deer hinds (*Cervus elaphus*) subjected to artificially shortened daily photoperiod or daily melatonin treatments. *Journal of Reproduction and Fertility* **73**, 255–260.

WELCH RAS, GURNSEY MP, BETTERIDGE K, MITCHELL RJ (1990) Goat fibre response to melatonin given in spring in two consecutive years. *Proceedings of the New Zealand Society of Animal Production* **50**, 335–338.

YEATES NTM (1955) Photoperiodicity in cattle. 1. Seasonal changes in coat character and their importance in heat regulation. *Australian Journal of Agriculture Research* **6**, 891–902.